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(57) Abstract

The instant invention relates to immunogenic synthetic peptides comprising amino acid sequences corresponding to antigenic determinants of the capsid of HAV, advantageously, linked directly or through a spacer molecule to carrier molecules suitable for vaccination of mammals.

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IMMUNOGENIC PEPTIDES

BACKGROUND OF THE INVENTION

Technical Field

The instant invention relates to vaccines for the prevention of hepatitis A viral (HAV) infection and, in particular, to vaccines comprising synthetic peptides having an amino acid sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the HAV capsid.

BACKGROUND INFORMATION

HAV accounts for 38% of the cases of viral hepatitis reported annually in the United States (D.P. Francis et al, Am. J. Med. 76:69-74 (1984)). On the basis of morphologic and biophysical characteristics, HAV has been classified among the Picornaviridae (I.D. Gust et al, Intervirology 20:1-7 (1983)). Like poliovirus, the virion is a naked icosahedron. The capsid contains at least three major structural proteins (VP1, VP2 and VP3), of which VP1 was previously believed to be the dominant surface protein (A.G. Coulepis et al, Intervirology 18:107-127 (1982)). Only a single antigenic specificity has been associated with HAV, and significant antigenic variation has not been recognized among different HAV strains (S.M. Lemon et al, Infect. Immun. 42:418-420 (1983)). Denaturation of the virus leads to a loss of antigenicity, suggesting that the relevant antigenic sites are strictly conformationally dependent (J.V. Hughes et al, J. Virol. 52:465-473 (1984)). By

covalently cross-linking monoclonal antibodies to intact HAV, one major antigenic site has been located on VP1 (J.V. Hughes et al, J. Virol. 52:465-473 (1984)).

Analysis of mutants that are resistant to neutralizing monoclonal antibodies has proven useful for identifying immunogenic sites involved in the neutralization of other picornaviruses (D.C. Diamond et al, Science 229:1090-1093 (1985); P.D. Minor et al, J. Gen. Virol. 65:1159-1165 (1985); B. Sherry et al, J. Virol. 53:137-143 (1985)). Genomic nucleotide substitutions associated with neutralization resistance, when analyzed in the context of the three-dimensional structure of poliovirus type 1 (PV1) or human rhinovirus type 14 (HRV14), have identified multiple discrete sites on the virion surface which appear to function both as immunogens and as attachment sites for neutralizing monoclonal antibodies (J.M. Hogle et al, Science 229:1358-1365 (1985); M.G. Rossman et al, Nature (London) 317:145-153 (1985)). Four such neutralization immunogenic sites have been identified on both PV1 and HRV14; they involve all three surface polypeptides (J.M. Hogle et al, Science 229:1358-1365 (1985); B. Sherry et al, J. Virol. 57:246-257 (1985)). In general, mutation occurring within one neutralization immunogenic site has conferred resistance to all monoclonal antibodies binding at that site, but not to those binding at alternate sites.

At present, no vaccine is available for prevention of infection with HAV. Because HAV closely resembles poliovirus and can be grown in cell cultures, intensive efforts over the past few years have been directed at the development of

inactivated and/or live attenuated HAV vaccines. For a variety of technical reasons (including difficulties in achieving high yields of virus in cell cultures), a clinically useful vaccine for prevention of infection with this virus has not yet been developed.

SUMMARY OF THE INVENTION

It is a general object of the instant invention to provide synthetic peptides that are capable of inducing the production in mammals of neutralizing antibodies against HAV.

It is another object of the invention to provide vaccines comprising a synthetic peptide(s) having an amino acid sequence corresponding to an antigenic determinant of the HAV capsid that is capable of inducing protective immunity in mammals against HAV.

It is an additional object of the invention to provide a method of detecting the presence of HAV antibodies in biological test samples.

These, and other objects which will be clear to those skilled in the art from the following detailed description, have been accomplished by providing synthetic peptides useful in producing an immunogenic response to the viral causative agents of HAV.

The invention relates to immunogenic preparations and vaccines made therefrom. Vaccines of the instant invention comprise a synthetic peptide(s) having an amino acid sequence corresponding to an antigenic determinant of the VP3 structural protein of the capsid of HAV, advantageously, linked to a suitable carrier molecule.

In one embodiment, the instant invention comprises a synthetic peptide having an amino acid sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the capsid of HAV, which peptide is capable of inducing in a mammal neutralizing antibodies against HAV. The synthetic peptide(s) of the instant invention corresponds to the region of VP3 containing the aspartate (asp)-70 residue.

In another embodiment, the instant invention comprises a DNA segment encoding the above-described peptides.

In a further embodiment, the instant invention comprises an immunogenic conjugate capable of inducing in a mammal neutralizing antibodies against HAV. The conjugate comprises a synthetic peptide having an amino acid sequence corresponding to an antigenic determinant of the VP3 structural protein of the capsid of HAV.

In yet another embodiment, the instant invention comprises a method of producing immunity to HAV comprising administering the above-described conjugate to a mammal.

In another embodiment, the instant invention comprises a method of detecting the presence of HAV antibodies in biological test samples.

BRIEF DESCRIPTION OF THE DRAWING

FIGURES 1A and 1B. Nucleotide sequence of VP1 of HM175 of HAV.

FIGURES 2A and 2B. Nucleotide sequence of VP3 of HM175 of HAV.

FIGURE 3. Competition between monoclonal antibodies for attachment to HAV fixed to a solid-phase support.

FIGURE 4. Map of genomic regions from various HAV neutralization-escape mutants sequenced by the dideoxynucleotide method.

FIGURE 5. Resistance of mutant S30 to neutralization by 9 different monoclonal antibodies to HAV.

DETAILED DESCRIPTION OF THE INVENTION

The instant invention relates to synthetic peptides, and DNA segments encoding same, corresponding to immunogenic epitopes of HAV and vaccines made therefrom. These novel immunogenic agents are prepared by synthesizing, either chemically or by recombinant techniques, peptides having an amino acid sequence corresponding to, or homologous with, B-cell epitopes present on the VP3 structural protein of the capsid of HAV. The peptides of the instant invention correspond to the region of VP3 containing the asp residue at position 70. The peptides, advantageously, linked to an appropriate carrier molecule, evoke the production in mammals of high titers of neutralizing antibodies against HAV. Vaccines thus formed are useful for immunization against HAV infection when administered to mammals, for example, intramuscularly, subcutaneously or intradermally, advantageously, intradermally.

To topologically map immunogenic sites on HAV which elicit neutralizing antibodies, eight neutralizing monoclonal antibodies were evaluated in competition immunoassays employing radiolabelled monoclonal antibodies and HM-175 virus according to procedures described in J. Virol., Vol. 61, No. 2, Feb. 1987, pp. 491-498 (the entire contents of which reference is hereby incorporated by reference and relied upon). Whereas two antibodies (K3-4C8 and K3-2F2) bound to overlapping epitopes, the epitope bound by a third antibody (B5-B3) was distinctly different as evidenced by a lack of competition between antibodies for binding to the virus. The other five antibodies variably blocked the binding of both K3-4C8--K3-2F2 and B5-B3, suggesting that these epitopes are closely spaced and part of a single neutralization immunogenic site. Several combinations of monoclonal antibodies blocked the binding of polyclonal human convalescent antibody by greater than 96%, indicating that the neutralization epitopes bound by these antibodies are immunodominant in humans.

Virus mutants resistant to monoclonal antibody-mediated neutralization have proven helpful in identifying immunogenic sites on other picornaviruses (D.M.A. Evans et al, Nature 304:459-462 (1983); P.D. Minor et al, J. Gen. Virol. 67:1283-1291 (1986); B. Sherry et al, J. Virol. 53:137-143 (1985)), but the application of this approach to characterization of HAV antigenic sites has been hampered by slow and inefficient virus replication in vitro. Most HAV isolates are noncytopathic, highly cell-associated, and demonstrate a substantial nonneutralizable fraction in vitro (S. M. Lemon, N. Engl. J. Med. 313:1059-

1067 (1985); J.R. Ticehurst, Seminars in Liver Dis. 6:46-55 (1986)). To overcome these problems, a radioimmunofocus assay was developed for HAV. The assay was based on the immune autoradiographic detection of virus replication foci developing beneath agarose overlays. The method was modified for clonal isolation of virus variants (S.M. Lemon et al, J. Clin. Microbiol. 17:834-839 (1983); S.M. Lemon et al, J. Virol. Methods 11:171-176 (1985)). A quantitative neutralization assay, based on the radioimmunofocus assay (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)), demonstrated that extraction of virus with lipid solvents substantially reduces the nonneutralizable virus fraction (S.M. Lemon et al, J. Gen. Virol. 66:2501-2505 (1985)). Nonetheless, most clonally isolated virus variants recovered from agarose overlays following stringent monoclonal antibody-mediated neutralization reactions demonstrated continuing neutralization susceptibility. The selection of spontaneous neutralization-escape mutants of HAV required a process of repeated neutralization and passage in the presence of antibody, followed by clonal isolation from agarose overlays (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)).

Neutralization resistance of such mutants is associated with reduced binding of antibody to the virion (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)). To identify capsid protein amino acid mutations associated with neutralization resistance, and by inference sites involved in antibody attachment on the virion surface, methods for RNA sequencing (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)) were modified to permit the

sequencing of as little as 2 to 10 ng of template RNA.

Mutant S30, selected by neutralization and passage in the presence of monoclonal antibody K2-4F2, showed no decrease in titer when incubated with this antibody (J.T. Stapleton et al, J. Virol. 61:491-498 (1987); see Table 1). The entire capsid-encoding region of S30 RNA was sequenced by primer-extension (see Example 2). Its sequence was then compared with that obtained from cDNA clones of parental virus with normal neutralization phenotype, and with the sequence of S32 virus. This variant was selected against monoclonal antibody B5-B3 but had substantially lost neutralization resistance during amplification in the absence of antibody.

The only mutation identified in the capsid-encoding region of the neutralization-resistant S30 virus was a G to C substitution at base 1677, predicting a change in asp-70 of capsid protein VP3 to his. This substitution also was present in two other mutants selected against K2-4F2, S18 and S27, but absent in mutant S20 which had reverted to neutralization susceptibility during final large volume cell culture passage (see Table 1). Because of the method of mutant selection (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)), these viruses are believed to represent sibling clones derived from a single neutralization-resistant parent. Asp-70 was unchanged in S32, and also in S1, S33, S34 and S57. The S1, S33, S34 and S57 mutants were initially selected for resistance to other antibodies but had reverted to neutralization susceptibility during passage in the absence of antibody. In variant S32, a C to T mutation was evident at base position 2512, predicting a change in ser-102 of VP1 to leu. S32

demonstrated significant neutralization susceptibility after amplification in the absence of antibody (see Table 1).

Table 1

Neutralization Resistance of HAV Variants

Cognate Antibody	Mutant	Neutralization Index[1]	Control Neutralization Index[2]
K2-4F2	S30	-0.09	1.33
	S18	-0.12	1.53
	S20	0.88	1.80
	S27	-0.06	1.53
K3-4C8	S1	0.92	1.66
B5-B3	S32	0.65	1.06
	S33	0.12	0.60
	S34	0.23	0.60
JC	S57	1.41	1.92

[1] Neutralization index = $\log_{10}$ reduction in virus titer following neutralization with the cognate antibody, determined by radioimmunofocus reduction assay (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983))

[2] Neutralization index of parent HM175 virus in same assay.

To determine whether the substitution at asp-70 of VP3 conferred resistance to other monoclonal antibodies (see Example 1), S30 was tested for neutralization resistance to nine other monoclonal antibodies, including B5-B3 (see Example 3). Although the degree of neutralization varied with different antibodies, S30 was relatively resistant to each. Neutralization resistance was most evident with monoclonal antibodies having activities similar to the cognate K2-4F2 in competition studies (813, 6A5, 10.09 and 1B9; see Example 1), but S30 was at least partially resistant to each of the antibodies tested.

Asp-70 of VP3 is, therefore, involved in the antigenic site recognized by each of these monoclonal antibodies, and is thus part of an immunodominant antigenic site of HAV. While the mutation at asp-70 of VP3 of HAV might hypothetically have resulted in capsid conformational changes preventing neutralization by antibodies binding at distant sites, such an interpretation conflicts with crystallographic evidence that mutations conferring neutralization resistance in poliovirus and rhinovirus cluster at specific surface sites most likely representing the relevant epitopes (M.D. Rossman et al, Nature 317:145-153 (1985); J.M. Hogle et al, Science 229:1358-1365 (1985)).

Given in FIGURES 1A and 1B is the nucleotide sequence of VP1 of HM175 strain HAV (circled residue is ser-102 of predicted amino acid sequence). Figures 2A and 2B show the nucleotide sequence of VP3 of HM175 strain HAV (circled residue is asp-70 of predicted amino acid sequence).

Synthetic peptides of the instant invention derived from the asp-70 region of VP3 comprise, for example, an amino acid sequence that includes IPTLAAQFPFNASDSVGQ, DSVGQQ, SDSVGQ, ASDSVG, NASDSV, FNASDS, PFNASD or a portion of any one of these sequences that is capable of inducing neutralizing antibodies.

Peptides of the invention can be synthesized either chemically (R.B. Merrifield, J.A.C.S., Vol. 83, 1963, pp. 2149-2154) or using recombinant techniques (B.E. Clarke et al, Nature, Vol. 330, 1987, pp. 381-384).

Carrier molecules to which the synthetic peptides of the instant invention can be covalently linked (conjugated) are, advantageously, non-toxic, pharmaceutically acceptable and of a size sufficient to produce an immune response in mammals. An example of a suitable carrier molecule is the hepatitis B core protein (B.E. Clarke et al, Nature, 330:381-384 (1987)). Other suitable carrier molecules well known in the art can also be used.

The synthetic peptides can also be administered with a pharmaceutically acceptable adjuvant, for example, alum.

Linkage of a carrier molecule to a synthetic peptide of the invention can be direct or through a spacer molecule. Spacer molecules are, advantageously, non-toxic and reactive. Suitable spacer molecules include those well known in the art.

Linkage of the carrier molecule to the synthetic peptide can be accomplished using a coupling agent. Advantageously, the heterofunctional coupling agent M-maleimidobenzoyl-N-hydroxysuccinimide ester or the water soluble

compound m-maleimido-benzoylsulfosuccinimide ester is used (Green et al, Cell 28:477 (1982)).

The present invention also relates to a vaccine against HAV infection comprising, in addition to the above-described synthetic peptides having an amino acid sequence corresponding to the region of VP3 containing the asp-70 residue (examples of such sequences being given above), an additional immunogenic sequence(s) from the HAV capsid which, advantageously, is a synthetic peptide having an amino acid sequence corresponding to the region of VP1 containing the ser-102 residue. Such synthetic peptides comprise, for example, a sequence of amino acids that includes TFTFNS, FTFNSN, TFNSNN, FNSNNK, NSNNKE, SNNKEY, or a portion of any one of these sequences.

The present invention also relates to a method of producing immunity to HAV in a mammal comprising administering to a mammal at least one of the above-described conjugates in an amount sufficient to induce the production of neutralizing antibodies against HAV.

In addition to being used as a vaccine or a component of a vaccine, the synthetic peptides of the instant invention can also be used for diagnostic purposes. Peptides of the instant invention can be used, for example, in standard enzyme linked immunosorbent assays or radioimmunoassays to detect the presence of HAV antibodies.

The following nonlimiting examples illustrate the invention in more detail.

Example 1

COMPETITION BETWEEN MONOCLONAL ANTIBODIES FOR ATTACHMENT TO HAV FIXED TO A SOLID-PHASE SUPPORT

Gradient-purified HM175 strain HAV was bound to polyclonal human convalescent antibody coating wells of a polyvinylchloride microtiter plate (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)) Dilutions of ten different murine monoclonal antibodies were added to the virus-coated wells prior to the addition of much smaller quantities of $[^{125}I]$-labelled monoclonal antibody K3-4C8 or B5-B3 (see ~~solid~~ diagonally stripped and stippled columns, respectively, in Figure 3). The quantity of radiolabelled antibody bound to virus was compared with the quantity bound in the absence of any competing antibody (see Figure 3). Results obtained with radiolabelled K3-4C8 were similar to those obtained with radiolabelled K2-4F2. The source of the monoclonal antibodies was as follows: K3-4C8, K3-2F2 and K2-4F2, Commonwealth Serum Institute, Melbourne, Australia (A. MacGregor et al, J. Clin. Microbiol. 18:1237-1243 (1983)); 6A5, 1B9, 2D2 and 3E1, J. Hughes and E. Emini, Merck Institute of Therapeutic Research, West Point, PA, USA (J.V. Hughes et al, J. Virol. 52:465-473 (1984)); 813 and 10.09, D. Crevat and E. Deloince, Clonatec, Paris, France; and B5-B3, R. Tedder, Middlesex Hospital, London, U.K.

Example 2

MAP OF GENOMIC REGIONS

The map of genomic regions from various HAV neutralization-escape mutants was sequenced by the dideoxynucleotide method (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)). The genomic region encoding the capsid proteins of HM175 strain HAV is depicted at the top of Figure 4, with nucleotide positions and putative peptide cleavage sites displayed according to Cohen et al (J.I. Cohen et al, J. Virol. 61:50-59 (1987)). At the right of Figure 4, the various HM175 variants included in this study (S1 through S57) are listed along with the parent virus HM175, while to the left are shown the antibodies against which these variants were selected. JC was polyclonal human convalescent antibody, while the other antibodies were murine monoclonal ascitic fluids. Solid lines indicate RNA regions sequenced, while vertical arrows indicate the location of identified nucleotide base substitutions. Neutralization-escape variants of HM175 virus were isolated as described previously (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)), and amplified in 850 cm^2 roller bottle cultures of BS-C-1 cells in the absence of antibody (to conserve antibody). Virus was assessed for continued neutralization-resistance or reversion to neutralization susceptibility at two weeks by radioimmunofocus reduction assay (see Table 1; J.T. Stapleton et al, J. Virol. 61:491-498 (1987); S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)) and harvested by freeze-thawing of cells at three

weeks. After purification of virus on hybrid sucrose-cesium chloride gradients (J. de Chastonay et al, Virology 157:268-275 (1987)), virus was treated with proteinase K/sodium dodecyl sulfate (J.R. Ticehurst et al, Proc. Nat. Acad. Sci. USA 80:5885-5889 (1983)) and viral RNA was extracted with phenol-chloroform. 2-10 ng purified viral RNA was annealed with 13.5 ng HAV-specific oligonucleotide primers and sequenced by reverse transcriptase-mediated primer-extension (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)) in the presence of dideoxynuclotides and high specific activity [^{35}S]-labelled deoxynucleotides (1000-1500 Ci/mole each). Reaction products were separated on 8% acrylamide, 7.6 M urea, Tris-borate-EDTA gradient gels. The sequence was compared with that derived from cDNA clones of cell culture-adapted HM175 virus.

Example 3

RESISTANCE OF MUTANT S30 TO NEUTRALIZATION BY NINE DIFFERENT MONOCLONAL ANTIBODIES TO HAV

The HAV variant S30 was selected by repeated neutralization and passage in the presence of antibody K2-4F2 (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)) and has a mutation at base position 1677 leading to substitution of asp-70 of VP3 with his. Neutralization resistance was assessed by the radioimmunofocus reduction method (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)) using a chloroform-extracted, 0.1% sodium lauryl sarcosinate-treated virus inoculum of

approximately 4000 radioimmunofocus-forming units/ml and a 10^{-1} or 10^{-2} dilution of antibody. Results are presented as $\log_{10}$ reductions in virus titer and are shown for S30 virus as stippled columns, and for plaque-purified parent HM175 virus as ~~solid~~ diagonally striped columns (see Figure 5). Previous studies demonstrated that S30 virus was also resistant to neutralization by K3-2F2 (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)).

The foregoing invention has been described in some detail by way of examples for purposes of clarity and understanding. It will be obvious to those skilled in the art from a reading of the disclosure that the synthetic peptides of the instant invention may differ slightly in amino acid sequence without departing from the scope of the invention. It will also be obvious that various combinations in form and detail can be made without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A synthetic peptide capable of producing an immunogenic response to the viral causative agent of hepatitis A comprising a chain of amino acids having a sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid, which peptide is capable of inducing in a mammal neutralizing antibodies against HAV.

2. The peptide according to claim 1, wherein said peptide comprises an amino acid sequence corresponding to a region of VP3 containing *asp*-70.

3. The peptide according to claim 2, which peptide comprises the sequence DSVGQQ.

4. The peptide according to claim 2, which peptide comprises the sequence SDSVGQ.

5. The peptide according to claim 2, which peptide comprises the sequence ASDVGV.

6. The peptide according to claim 2, which peptide comprises the sequence NASDSV.

7. The peptide according to claim 2, which peptide comprises the sequence FNASDS.

8. The peptide according to claim 2, which peptide comprises the sequence PFNASD.

9. The peptide according to claim 2, which peptide comprises the sequence IPTLAAQFPFNASDSVGQ.

10. An immunogenic conjugate capable of inducing in a mammal the production of high titers of neutralizing antibodies against HAV, said conjugate comprising:

(i) a carrier molecule covalently linked to (ii) a synthetic peptide consisting essentially of a chain of amino acids having a sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid containing *asp*-70.

11. The conjugate according to claim 10, further comprising a synthetic peptide having an amino acid sequence homologous to a portion of the amino acid sequence of an immunogenic site of the VP1 structural protein of the HAV capsid containing *ser*-102.

12. The conjugate according to claim 10, wherein said carrier molecule is hepatitis B core protein.

13. The conjugate according to claim 10, wherein said carrier molecule is covalently linked to said peptide through at least one spacer molecule.

14. A method of producing immunity to HAV in a mammal comprising administering to said mammal at least one conjugate according to claim 10 in an

amount sufficient to induce the production of neutralizing antibodies against HAV.

15. A method of determining the presence and titers in mammalian serum of neutralizing antibodies against HAV comprising the steps of:

(i) contacting a synthetic peptide consisting essentially of a chain of amino acids having a sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid containing *asp*-70, with antibodies from mammalian serum; and

(ii) measuring the formation of complexes between said peptide and said antibody.

16. A DNA segment encoding a sequence of amino acids homologous to a portion of the amino acid sequence of a neutratization immunogenic site of the VP3 structural protein of the HAV capsid, which pepide is capable of inducing in a mammal neutralizing antibodies against HAV.

FIG. 1A

LIMITS: 2207 3106

Nucleotide sequence VP1 of HM175 strain HAV; underlined residue is ser-102 in predicted amino acid sequence.

									2236										2266
GTT	GGA	GAT	GAT	TCT	GGA	GGT	TTT	TCA	ACA	ACA	GTT	TCT	ACA	GAA	CAG	AAT	GTT	CCA	GAT
V	G	D	D	S	G	G	F	S	T	T	V	S	T	E	Q	N	V	P	D
									2296										2326
CCC	CAA	GTT	GGT	ATA	ACA	ACC	ATG	AAA	GAT	TTG	AAA	GGA	AAA	GCT	AAC	AGA	GGG	AAA	ATG
P	Q	V	G	I	T	T	M	K	D	L	K	G	K	A	N	R	G	K	M
									2356										2386
GAT	GTT	TCA	GGA	GTA	CAA	GCA	CCT	GTG	GGA	GCT	ATC	ACA	ACA	ATT	GAG	GAT	CCA	GTT	TTA
D	V	S	G	V	Q	A	P	V	G	A	I	T	T	I	E	D	P	V	L
									2416										2446
GCA	AAG	AAA	GTA	CCT	GAG	ACA	TTT	CCT	GAA	TTG	AAA	CCT	GGA	GAA	TCC	AGA	CAT	ACA	TCA
A	K	K	V	P	E	T	F	P	E	L	K	P	G	E	S	R	H	T	S
									2476										2506
GAT	CAT	ATG	TCC	ATC	TAC	AAG	TTT	ATG	GGA	AGG	TCT	CAT	TTC	TTG	TGC	ACT	TTT	ACA	TTC
D	H	M	S	I	Y	K	F	M	G	R	S	H	F	L	C	T	F	T	F
									2536										2566
AAT	TCA	AAT	AAT	AAA	GAG	TAC	ACA	TTT	CCT	ATA	ACC	TTG	TCT	TCA	ACC	TCT	AAT	CCT	CCT
N	S	N	N	K	E	Y	T	F	P	I	T	L	S	S	T	S	N	P	P
									2596										2626
CAT	GGT	TTG	CCA	TCA	ACA	CTG	AGG	TGG	TTT	TTC	AAC	TTG	TTT	CAG	TTG	TAT	AGA	GGG	CCT
H	G	L	P	S	T	L	R	W	F	F	N	L	F	Q	L	Y	R	G	P

TO FIG. 1B

FROM FIG. IA

```
                                       2656                                                     2686
TTA GAT CTG ACA ATT ATT ATT ACA GGA GCA ACT GAT GTA GAT GGC ATG GCC TGG TTC ACT
 L   D   L   T   I   I   I   T   G   A   T   D   V   D   G   M   A   W   F   T

                                       2716                                                     2746
CCA GTA GGT CTT GCC GTT GAT ACT CCT TGG GTA GAG AAG GAG TCA GCT TTG TCT ATT GAC
 P   V   G   L   A   V   D   T   P   W   V   E   K   E   S   A   L   S   I   D

                                       2776                                                     2806
TAC AAA ACT GCT CTT GGA GCT GTC AGA TTT AAC ACA AGG AGA ACA GGG AAC ATT CAG ATT
 Y   K   T   A   L   G   A   V   R   F   N   T   R   R   T   G   N   I   Q   I

                                       2836                                                     2866
AGA TTA CCA TGG TAT TCT TAT TTA TAT GCT GTG TCT GGA GCA CTG GAT GGT TTG GGT GAC
 R   L   P   W   Y   S   Y   L   Y   A   V   S   G   A   L   D   G   L   G   D

                                       2896                                                     2926
AAG ACA GAT TCT ACA TTT GGA TTG GTT TCT ATT CAG ATT GCA AAT TAC AAT CAT TCT GAT
 K   T   D   S   T   F   G   L   V   S   I   Q   I   A   N   Y   N   H   S   D

                                       2956                                                     2986
GAA TAC TTG TCT TTT AGT TGT TAT TTG TCT GTC ACA GAA CAA TCA GAG TTT TAT TTT CCC
 E   Y   L   S   F   S   C   Y   L   S   V   T   E   Q   S   E   F   Y   F   P

                                       3016                                                     3046
AGA GCT CCA TTG AAC TCA AAT GCC ATG TTA TCC ACT GAA TCA ATG ATG AGC AGA ATT GCA
 R   A   P   L   N   S   N   A   M   L   S   T   E   S   M   M   S   R   I   A

                                       3076                                                     3106
GCT GGA GAC TTG GAG TCA TCA GTG GAT GAT CCT AGA TCA GAG GAA GAT AAA AGA TTT GAG
 A   G   D   L   E   S   S   V   D   D   P   R   S   E   E   D   K   R   F   E
```

FIG. IB

FIG. 2A

LIMITS: 1469 2206

Nucleotide sequence of VP3 of HM175 strain HAV; underlined residue is asp-70 in predicted amino acid sequence.

									1498										1528
ATG	ATG	AGA	AAT	GAA	TTT	AGG	GTC	AGT	ACT	ACT	GAG	AAT	GTG	GTG	AAT	CTG	TCA	AAT	TAT
M	M	R	N	E	F	R	V	S	T	T	E	N	V	V	N	L	S	N	Y
									1558										1588
GAA	GAT	GCA	AGA	GCA	AAG	ATG	TCT	TTT	GCT	TTG	GAT	CAG	GAA	GAT	TGG	AAA	TCT	GAT	CCG
E	D	A	R	A	K	M	S	F	A	L	D	Q	E	D	W	K	S	D	P
									1618										1648
TCC	CAG	GGT	GGT	GGG	ATC	AAA	ATT	ACT	CAT	TTT	ACT	ACT	TGG	ACA	TCT	ATT	CCA	ACT	TTG
S	Q	G	G	G	I	K	I	T	H	F	T	T	W	T	S	I	P	T	L
									1678										1708
GCT	GCT	CAG	TTT	CCA	TTT	AAT	GCT	TCA	GAC	TCA	GTT	GGT	CAA	CAA	ATT	AAA	GTT	ATT	CCA
A	A	Q	F	P	F	N	A	S	$\underline{D}$	S	V	G	Q	Q	I	K	V	I	P
									1738										1768
GTT	GAC	CCA	TAT	TTT	TTC	CAA	ATG	ACA	AAT	ACA	AAT	CCT	GAC	CAA	AAA	TGT	ATA	ACT	GCT
V	D	P	Y	F	F	Q	M	T	N	T	N	P	D	Q	K	C	I	T	A
									1798										1828
TTG	GCT	TCT	ATT	TGT	CAG	ATG	TTT	TGT	TTT	TGG	AGA	GGA	GAT	CTT	GTC	TTT	GAT	TTT	CAA
L	A	S	I	C	Q	M	F	C	F	W	R	G	D	L	V	F	D	F	Q
									1858										1888
GTT	TTT	CCC	ACC	AAA	TAT	CAT	TCA	GGT	AGA	TTA	CTG	TTT	TGT	TTT	GTT	CCT	GGC	AAT	GAG
V	F	P	T	K	Y	H	S	G	R	L	L	F	C	F	V	P	G	N	E

TO FIG. 2B

FROM FIG. 2A

```
                                    1918                                              1948
CTA ATA GAT GTT TCT GGA ATC ACA TTA AAG CAA GCA ACT ACT GCT CCT TGT GCA GTA ATG
 L   I   D   V   S   G   I   T   L   K   Q   A   T   T   A   P   C   A   V   M

                                    1978                                              2008
GAT ATT ACA GGA GTG CAG TCA ACT TTG AGA TTT CGT GTT CCC TGG ATT TCT GAC ACT CCT
 D   I   T   G   V   Q   S   T   L   R   F   R   V   P   W   I   S   D   T   P

                                    2038                                              2068
TAC AGA GTG AAC AGG TAT ACA AAG TCA GCA CAT CAG AAA GGT GAG TAC ACT GCC ATT GGG
 Y   R   V   N   R   Y   T   K   S   A   H   Q   K   G   E   Y   T   A   I   G

                                    2098                                              2128
AAG CTT ATT GTG TAT TGT TAT AAC AGA TTG ACC TCT CCT TCT AAC GTT GCT TCC CAT GTC
 K   L   I   V   Y   C   Y   N   R   L   T   S   P   S   N   V   A   S   H   V

                                    2158                                              2188
AGA GTG AAT GTT TAT CTT TCA GCA ATT AAC TTG GAA TGT TTT GCT CCT CTT TAT CAT GCT
 R   V   N   V   Y   L   S   A   I   N   L   E   C   F   A   P   L   Y   H   A

ATG GAT GTT ACT ACA CAA
 M   D   V   T   T   Q
```

FIG. 2B

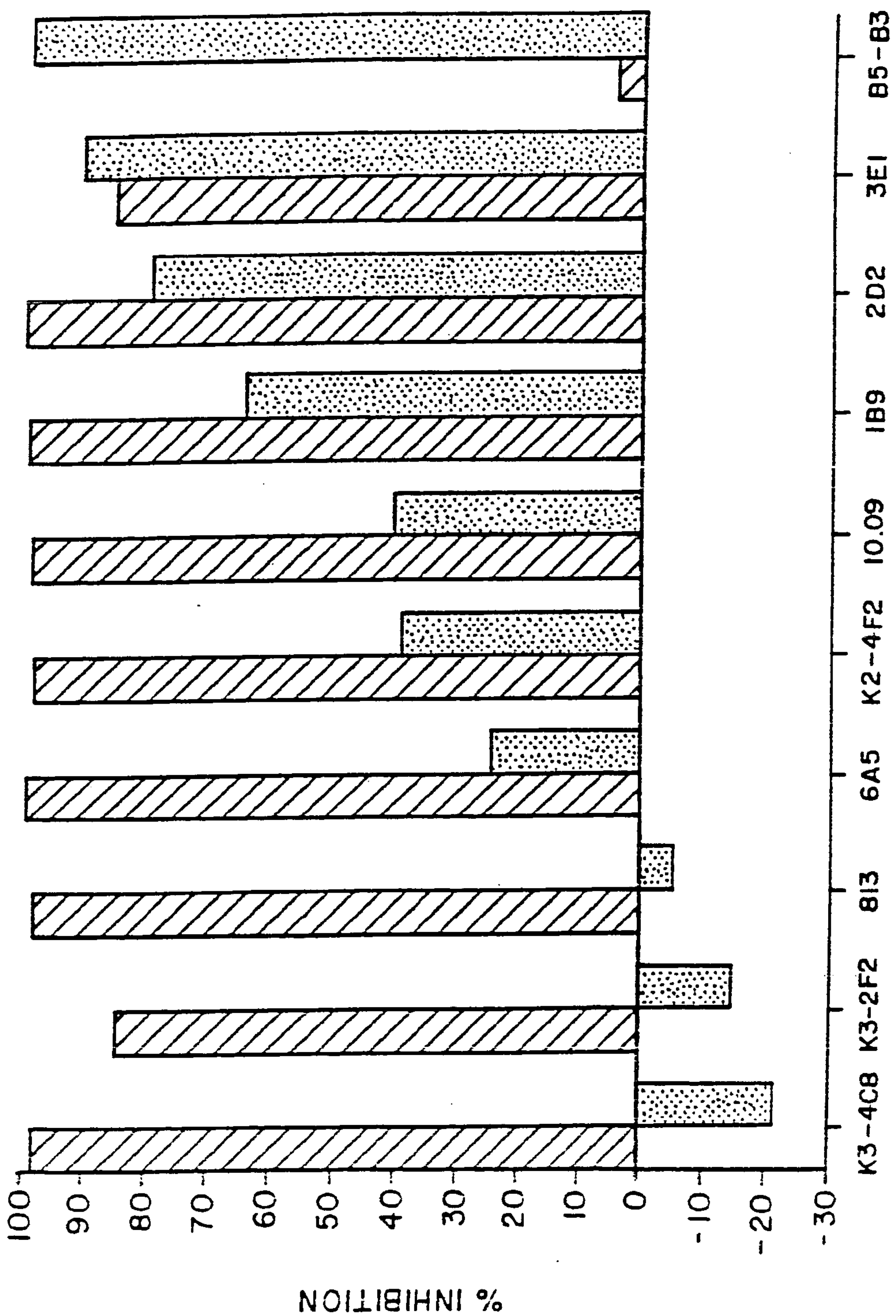
% INHIBITION
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
K3-4C8
K3-2F2
8I3
6A5
K2-4F2
10.09
IB9
2D2
3EI
B5-B3

FIG. 3

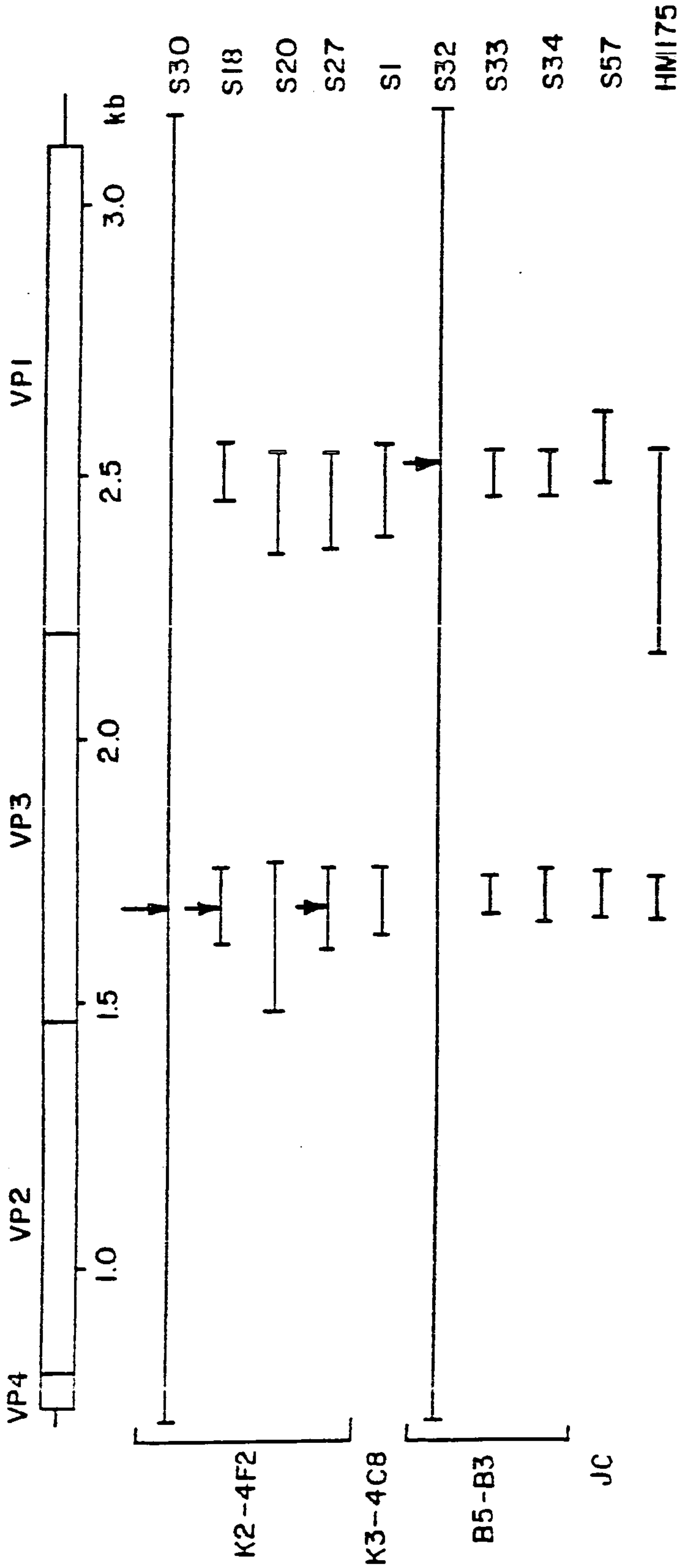
VP4
VP2
VP3
VP1
1.0
1.5
2.0
2.5
3.0
kb
S30
S18
S20
S27
S1
S32
S33
S34
S57
HM175
K2-4F2
K3-4C8
B5-B3
JC

FIG. 4

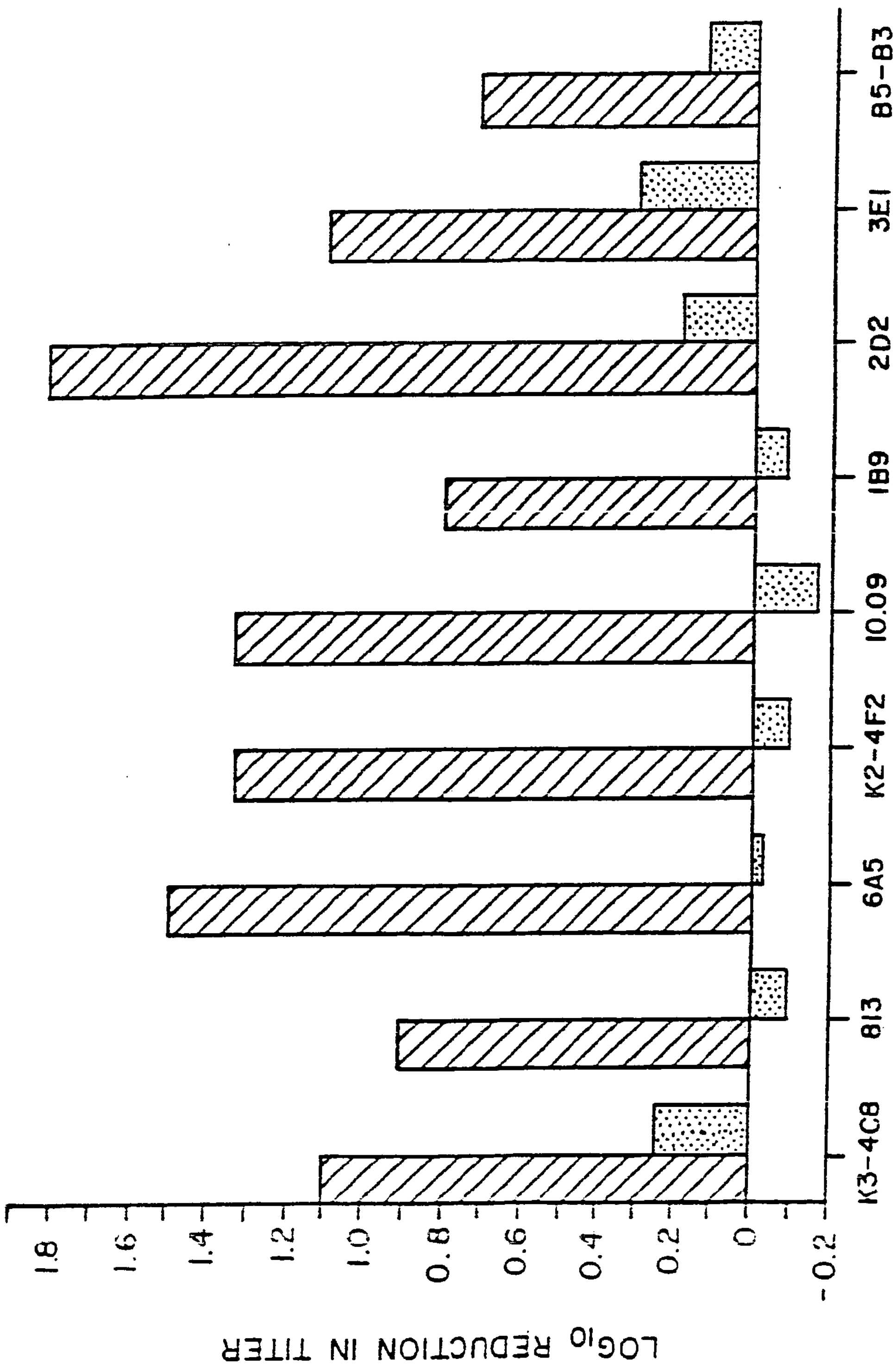
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
-0.2
LOG10 REDUCTION IN TITER
K3-4C8
8I3
6A5
K2-4F2
10.09
IB9
2D2
3EI
B5-B3

FIG. 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00097

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT. CL. 4th Ed. C07K 7/06,08,10; 13/00

U.S. CL.530/324,325,326,327,328,329,350;424/88;435/6,68

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/324,325,326,327,328,329,350;424/88;435/6,68

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Journal of General Virology, Vol. 57, Issued 1981 (Gunter)"The Physicochemical Properties of Infectious Hepatitis A Virions". pages 331-341 (See summary).	1-6
X	Journal of Virology, Vol. 52, No.2, Issued 1984 (Hughes)"Neutralizing Monoclonal Antibodies to Hepatitis A Virus:Partial Localization of a Neutralizing Antigenic Site". pages 465-473 (See summary).	1-16
X	Journal of Virology, Vol. 55, No. 2, Issued 1985 (Hughes)"Isolation and Immunizations with Hepatitis A Viral Structural Proteins: Induction of Antiprotein, Antiviral, and Neutralizing Resonses". pages 395-401 (See summary).	1-16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21 April 1989	08 JUN 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	DELBERT R. PHILLIPS

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	Journal Virology, Vol. 58, Issued 1986 (Wheeler) "Structure of the Hepatitis A Virion:Peptide Mapping of the Capsid Region".pages 307-313 (See summary).	1-16
X	Chemical Abstract, Vol. 102, Issued 24 June 1985 (Columbus, OH.) "Molecular cloning and partial sequencing of hepatitis A Viral cDNA". abstract no. 216279v (Linemeyer)	1-16
X,P	Chemical Abstract, Vol. 109, Issued 19 December 1988 (Columbus, OH.) "Identification of an immunodominant antigenic site involving the capsid protein VP3 of hepatitis A Virus". (Ping) Abstract no. 228155j.	1-16
X	Biological Abstract, Vol. 83, Issued 01 March 1987 "The genomic map of hepatitis A Virus: An Altermate analysis". (Diamond) Abstract no. 50184.	1-16
X	Biological Abstract, Vol. 77, Issued 01 February 1984 "Topology and immunoreactivity of capsid proteins in hepatitis A Virus". (Gerlich) Abstract no. 24102	1-16
X	US,A, 4,744,983 (Morein) Published 17 May 1988 (See Abstract).	1-16
X	US,A, 4,614,793 (Hughes) Published 30 September 1986 (See Abstract).	1-16
X	US,A, 4,578,269 (Morein) Published 25 March 1986 (See Abstract).	1-16